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A Classification of Virus Particles Based on Morphology

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"Virus, Virus, shining bright,
In the phosphotungstic night,
What immortal hand or eye,
Dare frame thy fivefold symmetry."
(With apologies to William Blake [1757-1827])

ABSTRACT

Recent improvements in electron microscope techniques which allow the study of virus fine structure have permitted the grouping of many viruses on a purely morphological basis. Briefly the techniques used in electron microscopy for the study of viruses are reviewed and the symmetry properties of virus particles as revealed by negative staining are discussed somewhat more fully.

Finally, virus particles are grouped on two bases, firstly the site of formation of the virus within the cell as seen by thin sectioning techniques, and secondly the symmetry property of the virus as seen by negative staining. Consideration of the groupings obtained in this way reveals that the biochemical and physical properties of a virus can be deduced from the readily established morphological characteristics.

OWING to new and improved methods for electron microscopy and improvements in the instrument itself, it is now possible to use morphological differences as one basis on which virus particles can be classified. This communication presents such a classification, but the important steps which made this possible will first be reviewed briefly.

The way in which virus particles were initially prepared for examination with the electron microscope was simply by allowing a drop of an aqueous virus suspension to dry on to a grid. Contrast, which permitted the outline of particles to be determined, was achieved because the virus particles produced greater scattering of the electrons than the areas between them. By this technique Kauschi *et al.* showed in 1939 that tobacco mosaic virus had a rod-like form. Next, the basic outline of several plant viruses was established by Stanley and Anderson in 1941; in every instance these were either rod-like or spherical. In 1942 Green *et al.* by this same technique showed that vaccinia virus had a brick-like form. Influenza virus was seen by Taylor *et al.* in 1943 and the Shope papilloma virus by Sharp *et al.* in 1942.[1]

A distinct advance occurred in 1945 when Williams and Wychoff devised a method whereby a metallic mist could be directed from a point source and at a known angle on to particles prepared as above. The shadows thus cast were devoid of metallic deposit. By knowing the angle from which the metal was sprayed and from studying the size and form of the shadows that were cast by different virus particles, the height and form of their upper surfaces could be established. This technique, called shadow casting, therefore enlarged the scope of the study of virus particles to three dimensions (Fig. 1a and b).[1]

The next great advance entailed the development of methods whereby sections of virus-infected tissues could be cut thin enough to be studied effectively with the electron microscope. It was less than a decade ago when this became possible, and it was owing to the evolution of microtomes that could cut extremely thin sections, the availability of embedding media that would hold the thin sections together and yet not cause distortion, the development of fixation methods that gave superior preservation, and the use of cutting edges of great sharpness. With these advances it became possible to see virus particles *in situ* in thin sections

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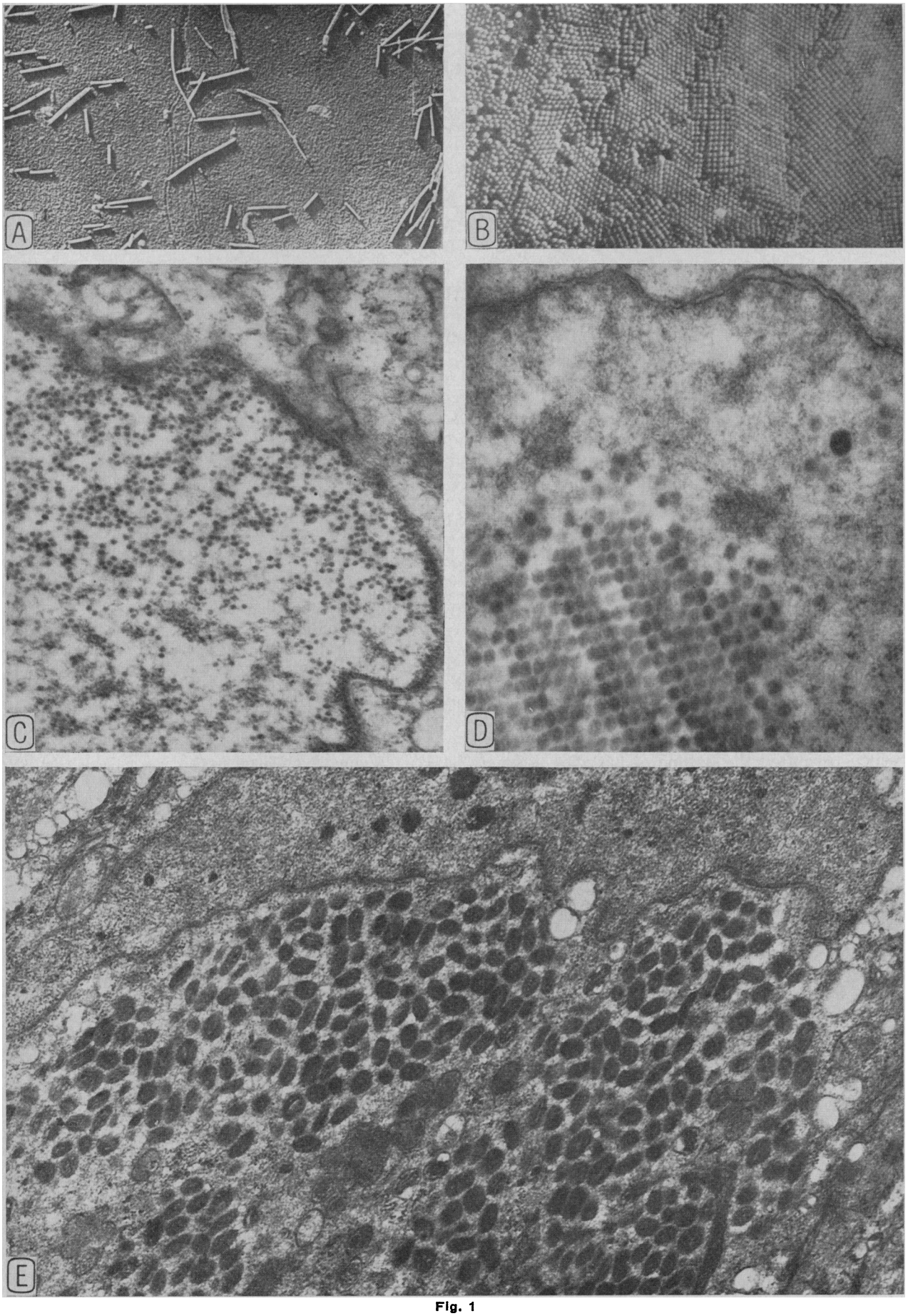

Fig. 1

of cells (Figs. 1 c to e).[1] The greatest use of this technique from the present point of view is to establish the location of the virus within the cell.

The most recent important advance in methods for studying viruses with the electron microscope occurred in 1959 when Brenner and Horne[2] applied the method of negative staining to virus particles. This method entails the treatment of virus suspensions with a solution of some electron-dense substance, usually phosphotungstic acid. The negative stain not only penetrates between all the particles in a preparation, but also into the most minute irregularities on their surfaces; and hence, since the electron beam penetrates sites where the phosphotungstic acid is *not* present, the surface configuration of particles is thrown into sharp relief. Adenovirus was one of the first to be visualized by this method. This was done by Horne *et al.*[3] and it showed the surface of the particles to be studded with projecting subunits that were geometrically arranged. This finding, as will be shown, opened the door to establishing morphological differences between different viruses, differences that were hitherto undisclosed, and, as we shall see, it was to provide a new means of classifying viruses on a morphological basis.[4, 13]

At first the method of negative staining was applied only to purified suspensions of virus. Since purification procedures may injure or even destroy virus particles, the use of purified suspensions limited to some extent the usefulness of the negative staining technique. However, methods developed by Almeida and Howatson[5] and by Parsons[6] at the Ontario Cancer Institute have overcome many of the difficulties involved with purified suspensions because their methods permit negative staining to be utilized *directly* on preparations of infected material without any prior purification procedures being required. These methods have the advantage of not only subjecting virus particles to the least possible stress,[7] but also permitting virus particles to be visualized *in situ*. For example, Fig. 7b and Fig. 7c were obtained by the first of these methods.[5]

How Morphology Involves Symmetry

To proceed further with establishing a morphological basis for classifying viruses it is necessary to delve more deeply into the nature and arrangements of the subunits which cover their surfaces. To do this involves a discussion of symmetry and studies of the symmetry of virus particles that were previously made by means of x-ray diffraction on crystals of virus particles (part of a crystal of poliovirus is shown in Fig. 1b).

In 1956, Crick and Watson,[8] chiefly from x-ray diffraction studies which they and others had made on purified virus particles, predicted that simple viruses would all be found to be composed of a centrally placed nucleic acid fraction and an external protective protein coat or shell. They predicted furthermore that the nucleic acid content of a virus particle would be too limited to be able to code information for the synthesis of a variety of proteins and hence that the protein shell of a virus particle would be found to be composed of small identical subunits of protein arranged identically on its surface. They predicted moreover that only two types of geometric arrangement would occur. Firstly, there would be one type in which the identical subunits would be arranged in the form of a helix, with the particle displaying helical symmetry. Secondly, in the instance of the so-called spherical viruses, the cubic symmetry found by x-ray diffraction in crystals of these viruses would extend to the individual virus particles themselves; each virus would have subunits arranged in cubic symmetry and this would mean that the form of the particles would be based on either the tetrahedron, octahedron, or icosahedron. The advent of the negative staining of virus particles made it possible to prove these predictions correct.

Since x-ray diffraction studies on purified virus crystals were a very important factor in leading to the predictions that virus particles, like crystals, would manifest symmetry, it was only to be expected after the advent of negative staining that some of the terminology commonly used in connection with crystals would be applied to virus particles. In particular, since different crystals display different types of symmetry and are classified to some extent by the kind of symmetry that they display, many viruses can now be classified as to whether they display cubic or helical symmetry.[9] What is meant by cubic symmetry in viruses will next be described.

Cubic Symmetry

In 1957 Williams and Smith showed that when tipula iridescent virus was shadow-cast from two different points, the contours of the shadows seen were those that would be thrown by an icosahedron;[10] this was the first verification by electron microscopy of one of Crick and Watson's predictions.[8]

The icosahedron is one of a class of solid geometrical bodies, the regular polyhedra, each type of which possesses the characteristics of having many faces all of which are identical with each other. The icosahedron has 20 faces and 12 vertices. As is shown in the accompanying diagrams (Fig. 2), it is possible to visualize such a body having

Fig. 1a.—A shadow-cast preparation of tobacco mosaic virus showing the rod-shaped particles. × 30,000. (Courtesy of Mr. L. Pinteric.) 1b.—A shadow-cast preparation of crystallized poliovirus. It is this ability to form crystals that made x-ray diffraction studies of viruses possible. × 36,000. (Courtesy of Mr. L. Pinteric.) 1c. Thin section preparation showing part of a cell infected with polyoma virus. Since this is a nuclear virus the particles are seen within the nucleus of the cell. × 35,000. 1d.—Another nuclear virus, Adenovirus Type 3. The micrograph shows part of a crystalline array of virus particles within the nucleus. The double nuclear membrane runs across the top of the micrograph. × 47,000. 1e—Thin section preparation of a cytoplasmic virus, in this case molluscum contagiosum. The nucleus of the cell is compressed to one side and the cytoplasm contains many pox-type particles. × 25,000.

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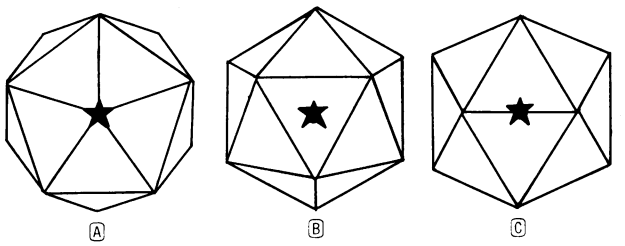

Fig. 2

Fig. 2.—Three different views of an icosahedron with the location of the axis of symmetry marked with a star in each case. The viewer is looking along such axes. **2a.**—Here the icosahedron is oriented so that it can be rotated around the axis to give five identical positions. This then is the axis of five-fold symmetry. **2b.**—The orientation in which the icosahedron can be rotated around the central axis to three identical positions, the axis of three-fold symmetry. **2c.**—In this position the icosahedron has only two identical positions, the one shown and that resulting from a rotation of 180° around the axis. This axis is the axis of two-fold symmetry.

three different central axes around which it could rotate. In rotating around the first axis (Fig. 2a) it could be stopped in five different positions in which its aspect would have identical appearances, and at the same time each half of the whole icosahedron would be identical to the other half. Hence in turning around this axis there would be five positions in which it would manifest symmetry. This axis is referred to as the axis of five-fold symmetry. If the axis were as shown in Fig. 2b, it would be possible to rotate the icosahedron to three places, which would all present the same aspect to a viewer. If the axis were as shown in Fig. 2c, an identical appearance would be observed in only two positions. Accordingly, since there is one axis about which an icosahedron can be rotated so that in five different positions it shows the same symmetrical aspect to the viewer, and another axis about which it can be rotated to show the same symmetrical aspect in three positions, and another, in two positions, an icosahedron is said to possess 5:3:2 axial symmetry. Experimental procedures have now shown that a large class of viruses possess this type of symmetry. It is customary to say that these viruses exhibit *cubic* symmetry. (The adjective *cubic* used in this way has a special meaning which is derived from the fact that a cube has four body diagonals, each one of which has a special relation to the others and could serve as an axis about which the cube could be rotated and manifest three-fold symmetry. Since the tetrahedron, the octahedron and the icosahedron all have at least one set of four axes that have a special relation to one another and about which they can be rotated to three different positions where they manifest symmetry, they are all said to possess cubic symmetry.[11]) Examples of viruses showing cubic symmetry are shown in Fig. 3.

Since it has now been shown by negative staining that many of the viruses that appear spherical in thin sections are actually icosahedral in form, we may now inquire whether or not negative staining has revealed any proof for the prediction of Crick and Watson to the effect that the protein coats of virus particles would be built of small identical subunits arranged in an identical fashion with one another. In considering this matter we shall run into the difficulty that the term subunit, in relation to virus particles, has been used in two ways and this requires some clarification.

With negative staining the protein coat of icosahedral virus particles is seen to be studded with extremely minute projections; these have been termed capsomeres. The capsomeres are the *morphological* subunits, and they fit together to cover the whole of the particle. The most efficient and economical shape for subunits to have, if they are to be fitted together to cover a flat surface, is that of a hexagon, as in a honeycomb.[12] The surface of an icosahedron presents a problem in this connection, for its covering must extend over vertices. However, the problem would be solved if each capsomere that was directly over a vertex was a pentagon, for hexagons could then be fitted against each of their five sides and so the protein shell would maintain an icosahedral form. An illustration of this concept is depicted in Fig. 3e, in which the central white subunit is a pentagon forming one of the vertices of an icosahedron. This arrangement of hexagonal and pentagonal capsomeres seems to hold for the whole series of icosahedral viruses that have as yet been identified (Fig. 4); the simplest virus of this series, bacteriophage ϕx174 (Fig. 3a), has 12 capsomeres and the largest number of subunits is found on tipula iridescent virus which has 812 of them.

Two variations of the arrangement of hexagons and pentagons described above should now be considered. The first relates to how the form of a virus of this type would be affected if for some reason pentagonal capsomeres were not present in their proper numbers and as a consequence hexagons were fitted together more extensively than in

Fig. 3.—This plate shows examples of negatively stained cubic viruses all at a magnification of × 300,000. **3a.**—Bacteriophage ϕx174 has the simplest arrangement of subunits of viruses showing cubic symmetry. It has 12 subunits, one placed at each vertex of an icosahedron. **3b.**—Turnip yellow mosaic is the only virus known at present that belongs to the series having a subunit placed centrally on each of the 20 triangular faces of an icosahedron; these together with the 12 subunits on the vertices of the icosahedron give a total of 32. **3c.**—While Fig. 3b shows the normal negative-stained appearance of the turnip yellow mosaic, this Figure (3c) has been photographically reversed to give greater prominence to the subunits. (Courtesy of Dr. H. E. Huxley.) **3d.**—A group of negatively stained wart-virus particles. Wart virus belongs to the papova group of viruses and there is at present controversy as to whether this group has 42 or 92 subunits. **3e.**—A model constructed of hexagons and pentagons according to the plan shown in Fig. 2. There are 30 white hexagons and 12 white pentagons representing the arrangement of capsomeres that would be present on a virus with 42 subunits. This may well be the arrangement for wart virus, shown in Fig. 3d of this plate. **3f.**—A tubular form of polyoma virus showing what happens when an icosahedral virus is incapable of forming "pentagons". Also of interest are the subunits that have detached from the top of the tubule as they remain in a hexagonal form. **3g.**—The arrow indicates a particle of Adenovirus Type 12 that clearly exhibits the icosahedral shape of the virus. By counting the number of subunits lying between two vertices it is possible to calculate the number of subunits composing the protein shell. **3h.**—Varicella virus, an example of the compound cubic group of viruses. The geometrically arranged capsid is seen less clearly as it is covered by an outer fringed membrane derived from the cell.

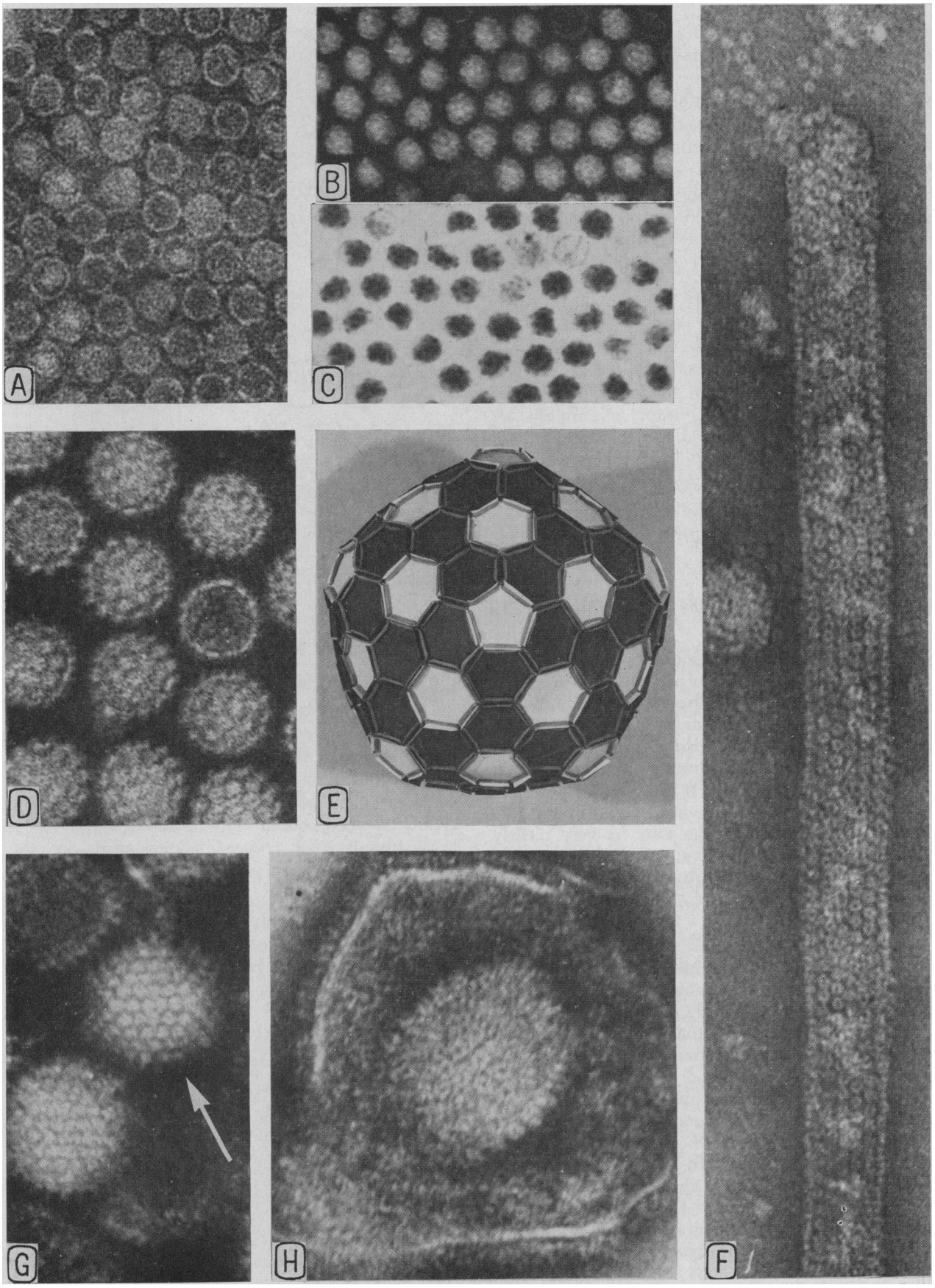

Fig. 3

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the usual particle. Without pentagons to cover vertices the particles could be visualized as continuing to increase in some direction by adding more hexagons in the region where the pentagons were missing. It is possible that this is the explanation for the fact that certain viruses[13] that are ordinarily icosahedral sometimes manifest tubular forms, as shown in Fig. 3f.

The other variation is in the arrangements of hexagons alone. As pointed out by Caspar and Klug,[9] only two arrangements are geometrically possible. Basically they are distinguished by the presence or absence of a hexagon placed centrally on each triangular facet of the icosahedron (Fig. 4). So far, only one virus, turnip yellow mosaic (Fig. 3b), has been shown to have hexagons placed centrally on the triangular facets.[14] Each of the two arrangements described gives rise to a series (Fig. 4) depending on the number of subunits between any two vertices, and the total number of subunits covering the particle can be calculated by the use of two simple formulae:

(a) $10(n-1)^2 + 2$, for the more common series which have no central subunits on their triangular facets,

and

(b) $30(n-1)^2 + 2$, for the series with centrally placed subunits,

where n = the number of subunits on one side of a triangle. For practical purposes this means that if it is possible to count the number of subunits between any two vertices, or, as it is more usually described, five-fold axes, then it is possible to calculate the number of subunits or capsomeres on the protein shell or capsid. It may be of interest to study the particle of adenovirus shown in Fig. 3g. The icosahedral form of the virus is very clearly shown, and it should be possible for the reader to count the number of subunits lying between vertices and hence estimate the number of capsomeres on the virus.

Since the morphological subunits, the capsomeres, of virus particles with cubic symmetry are either hexagons or pentagons, they are not, of course, identical. Accordingly if Crick and Watson's prediction[8] of identical protein subunits identically arranged is to hold, there must be smaller protein subunits than capsomeres and those "hexagons" that have broken off from the tube form in Fig. 3f do appear to be made up of even smaller units. These, it is suggested, could be the corner posts of the hexagons and pentagons. In Fig. 4 the hexagons and pentagons are shown with knobs at each corner and it is apparent that if these are the basic unit each subunit is now arranged identically with those around it. Each basic or structural unit is shown as having two strong bonds and one weak bond (Fig. 4); this concept of strong and weak bonds is based on the observation that, if disrupted, the subunits remain either in the hexagonal or pentagonal form as is shown in Fig. 3f. In view of these observations and this reasoning it is feasible to consider that the capsid of viruses showing cubic symmetry need contain only one basic type of protein subunit, that represented in effect by the corner posts of the pentagons or hexagons.

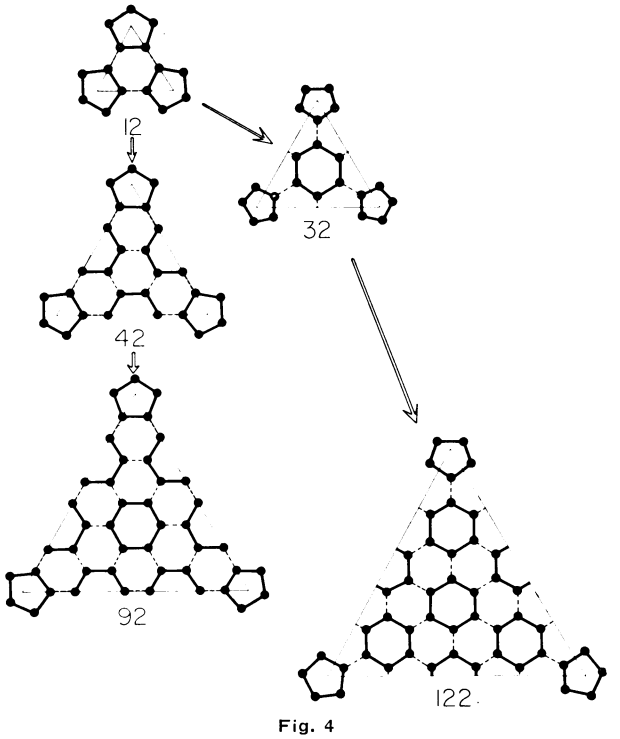

Fig. 4

Fig. 4.—A diagrammatic representation of the close-packed array of hexagons and pentagons on the surface of virus particles having cubic symmetry. The series 12, 42, 92, - - - - - 812 is the one that has most frequently been found. The diagram shows the arrangement of subunits on only one triangular facet of the icosahedron, but the relationship of the triangular facet to the whole icosahedron can be seen by comparing this to Fig. 2. It should be noted that "pentagons" are present only at vertices. The basic structural unit of these viruses is represented by the corner posts of the hexagons and pentagons and each one of these is identically arranged with regard to every other one.

Viruses such as those we have been discussing are designated as simple cubic viruses and consist simply of nucleic acid and protein. Some other viruses showing cubic symmetry, however, add an

Fig. 5.—This plate illustrates negatively stained helical viruses all at a magnification of × 300,000. **5a.**—Negatively stained rods of tobacco mosaic virus. The central hole of the helix is clearly seen but the spacing between the turns of the helix 23 A° is such that the actual helix is not resolved in untreated virus. **5b.**—A particle of Parainfluenza III. This virus belongs to the compound helical group and closely resembles measles and mumps virus. The outer membrane is covered with fine filamentous projections and at one point, indicated by an arrow, a short length of the internal helical component of diameter 170 A° can be seen. Although it is not completely established, the helix of viruses of this type would seem to be a single one. **5c.**—A small portion of the helical component from a completely disrupted measles virus particle. **5d.**—A second type of cytoplasmic compound helical virus, influenza. Three of the pleomorphic particles can be seen in the upper part of the micrograph. In contrast to the type of virus shown in Fig. 6b influenza has coarser projections on the outer membrane and does not disrupt spontaneously, having to be treated with a solvent such as ether before the 90 A° diam. internal helix can be seen. **5e.**—Internal component of rabies virus. The outer membrane of the complete virus is similar to that shown for parainfluenza in Fig. 5b; however, in this case the internal helix has a 90 A° diameter and is double in nature. The arrows indicate two points where the complete helix can be seen to divide into two separate filaments.

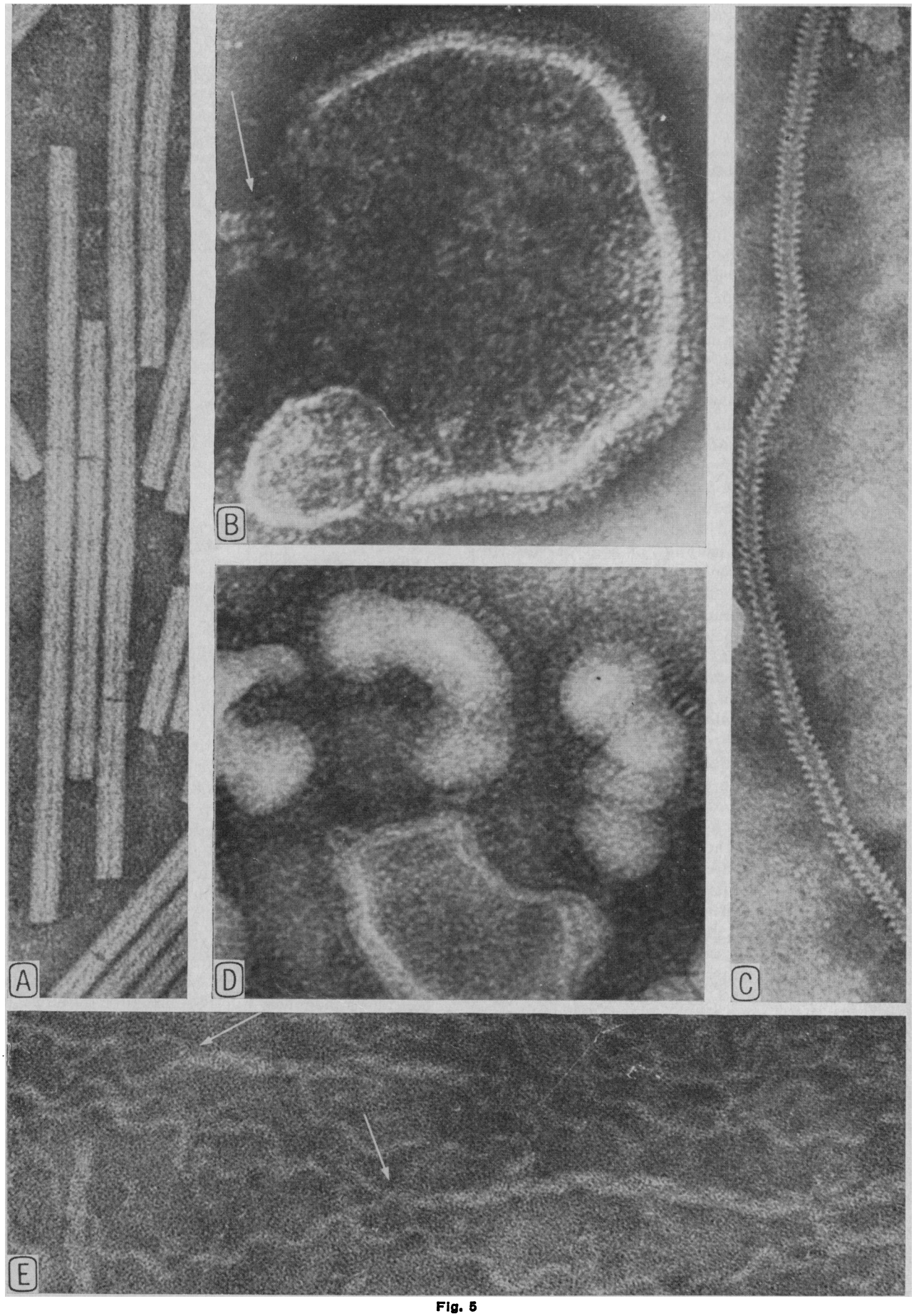

Fig. 5

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outer membrane at some stage of their development and these we shall call compound cubic viruses (Fig. 3h).

VIRUSES HAVING HELICAL SYMMETRY

A second and very important group of viruses can now be distinguished because they possess a morphological character in common, that of having helical symmetry. One of the first studied and best known of these is tobacco mosaic virus, which appears rod-shaped both by shadow-casting (Fig. 1a) and by negative staining (Fig. 5a). Although the virus appears rod-shaped by these methods, it actually is in the form of a helix; this has been shown in the electron microscope by the fact that if the virus is completely degraded the protein component can be reconstituted, and when this is done the helical nature of the rod becomes apparent because the helix in this instance is not so tightly wound.[15] Caspar's diagrammatic representation of the substructure of tobacco mosaic virus is shown in Fig. 6. This shows that the "rod" is composed of identical asymmetric protein subunits arranged in a helix.[9] In this arrangement each subunit except for one at each end is identically related to every other subunit. The ribonucleic acid of the virus lies in an inner groove and the efficiency of the protective protein shell is shown by the resistance of the virus to ribonuclease and by the fact that the virus is more heat-stable than RNA isolated from the virus.[9] Many plant viruses are similar in form to tobacco mosaic virus but differ from it in helix diameter and in some instances in flexibility.[4] All these are designated as simple helical viruses.

Some of the more complex animal viruses also exhibit helical symmetry. The difference between these and the simple ones described above is that although, like the simple helical viruses, their nucleic acid protein complex is in the form of a helix, they have in addition a surrounding membranous sac that is derived from the cell that the virus infected.[16] Viruses in this category we term compound helical viruses.

There are three groups of compound helical viruses known at present. These are distinguished from one another by (1) whether the helix is single or double, (2) the diameter of the helix, and (3) the nature of the projections of the outer membrane of the particle. Examples of all three are shown in Figs. 5a-e.

COMPLEX VIRUSES

On morphological grounds there is a third group of viruses that present either a more complicated or less well understood structure than those that are classed as having cubic or helical symmetry. At present these are classified as complex viruses. However, even in this group elements of either cubic or helical symmetry, or both, are present.

First to be considered here are the sophisticated tailed phages. In the T2 phage the head is described as a hexagonal prism with two hexagonal pyramids while the tail shows helical symmetry (Fig. 7a). The head of the *B. megatherium* phage is icosahedral.[4, 17]

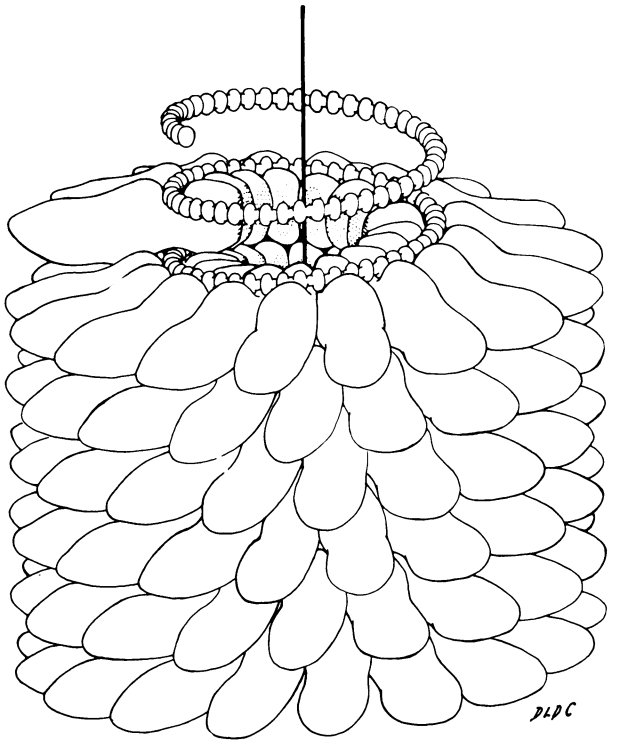

Fig. 6

Fig. 6.—A diagrammatic representation of tobacco mosaic virus. The asymmetric subunits are arranged identically with each other. The nucleic acid is shown as the smaller helix contained in a groove of the structural protein units. (Courtesy of Dr. D. L. D. Caspar.)

Pox viruses with negative staining exhibit an appearance similar to an untidy ball of wool (Fig. 7b), and as yet no definite symmetry properties have been resolved in them, although Horne *et al.*[3] have shown a helical structure in orf virus which belongs to this group,[18] and more recently Friedman-Kien, Rowe and Banfield[19] have shown a similar helical structure in a pox virus isolated from milker's nodules.

A third complex virus is vesicular stomatitis virus, the only known asymmetric animal virus.[20] This virus appears to contain some kind of internal helical arrangement but is sufficiently different in other respects to warrant its being placed in the complex category (Fig. 7c).

Fig. 7.—This plate comprises examples of negatively stained viruses that have been described as having complex morphology. All are shown at a magnification of × 300,000. **7a.**—Micrograph of T4 bacteriophage. The lower particle shows the normal appearance of the virus. The helical nature of the tail sheath is clearly visible. In the virus which is partially shown in the upper part of the micrograph the sheath is contracted and the inner stem which is used to "inject" the nucleic acid content of the head into the bacterium revealed. **7b.**—A negatively stained vaccinia virus particle still contained within a vesicle in the cell. No definite symmetry is associated with vaccinia virus although other members of the pox group have been shown to have a helical arrangement. **7c.**—At the upper and lower right of the micrograph, mature, bullet-shaped particles of vesicular stomatitis virus are seen. The surface of the virus is covered by fine filamentous projections. The incomplete budding form of the virus shows an internal helical arrangement.

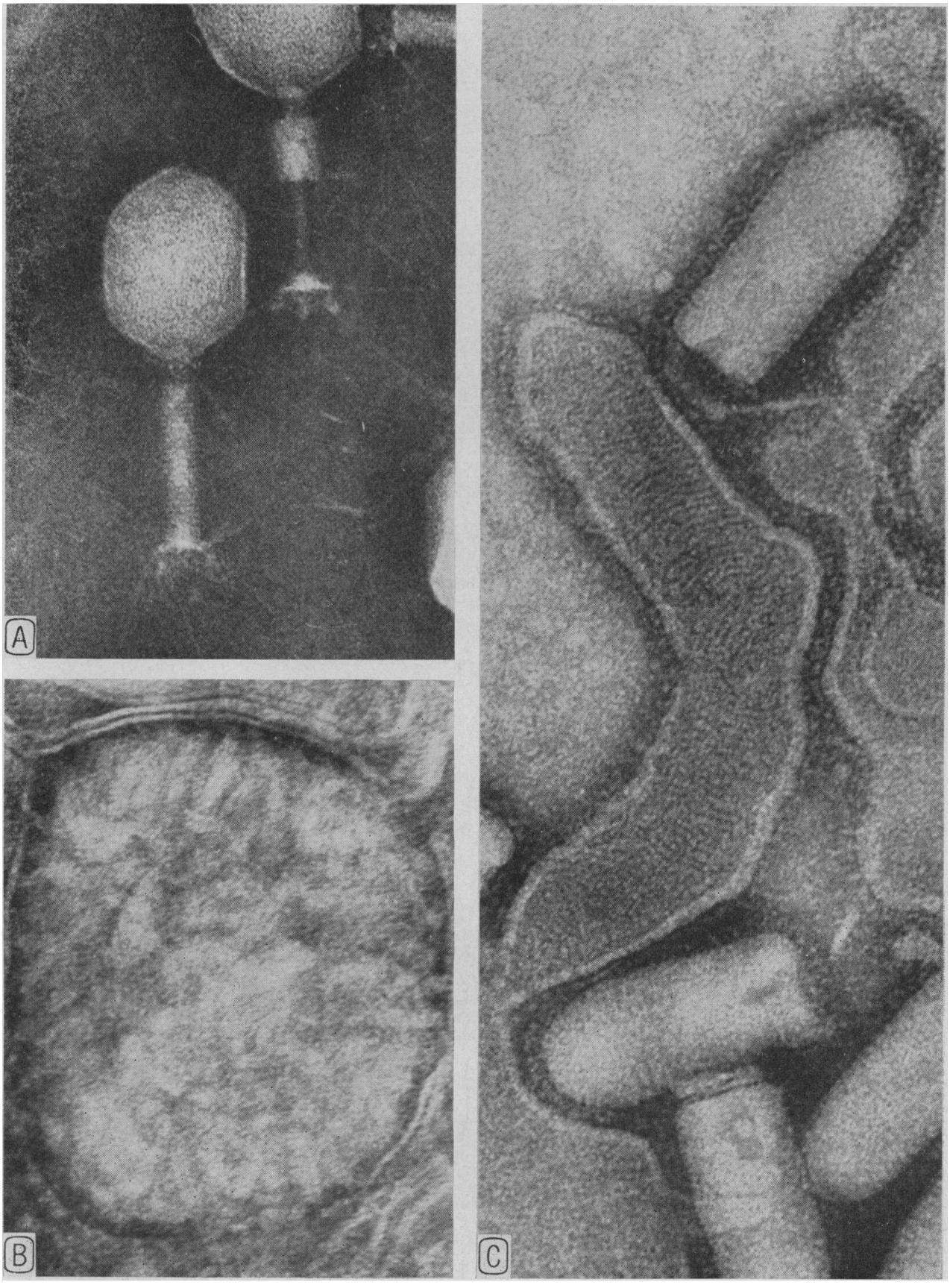

Fig. 7

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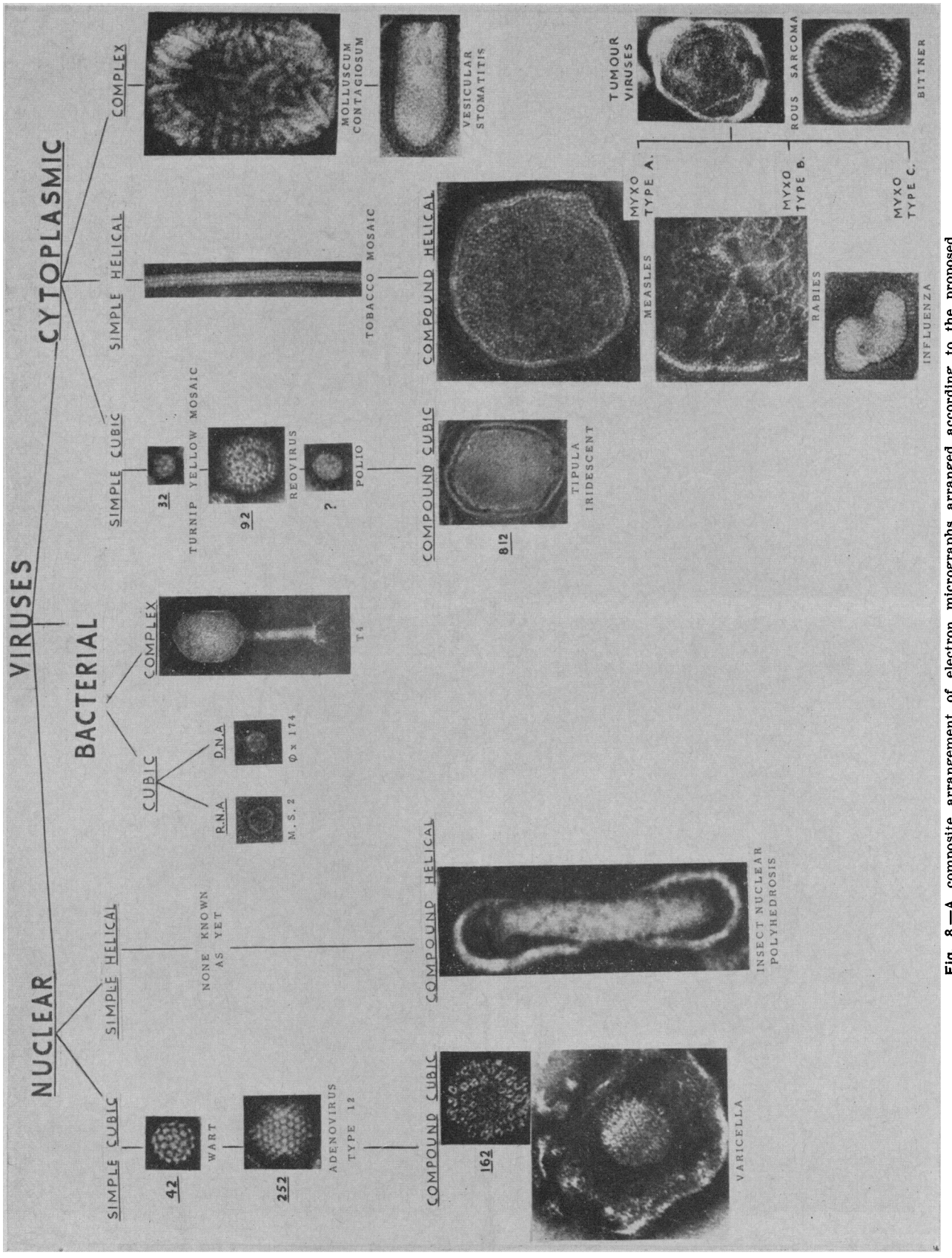
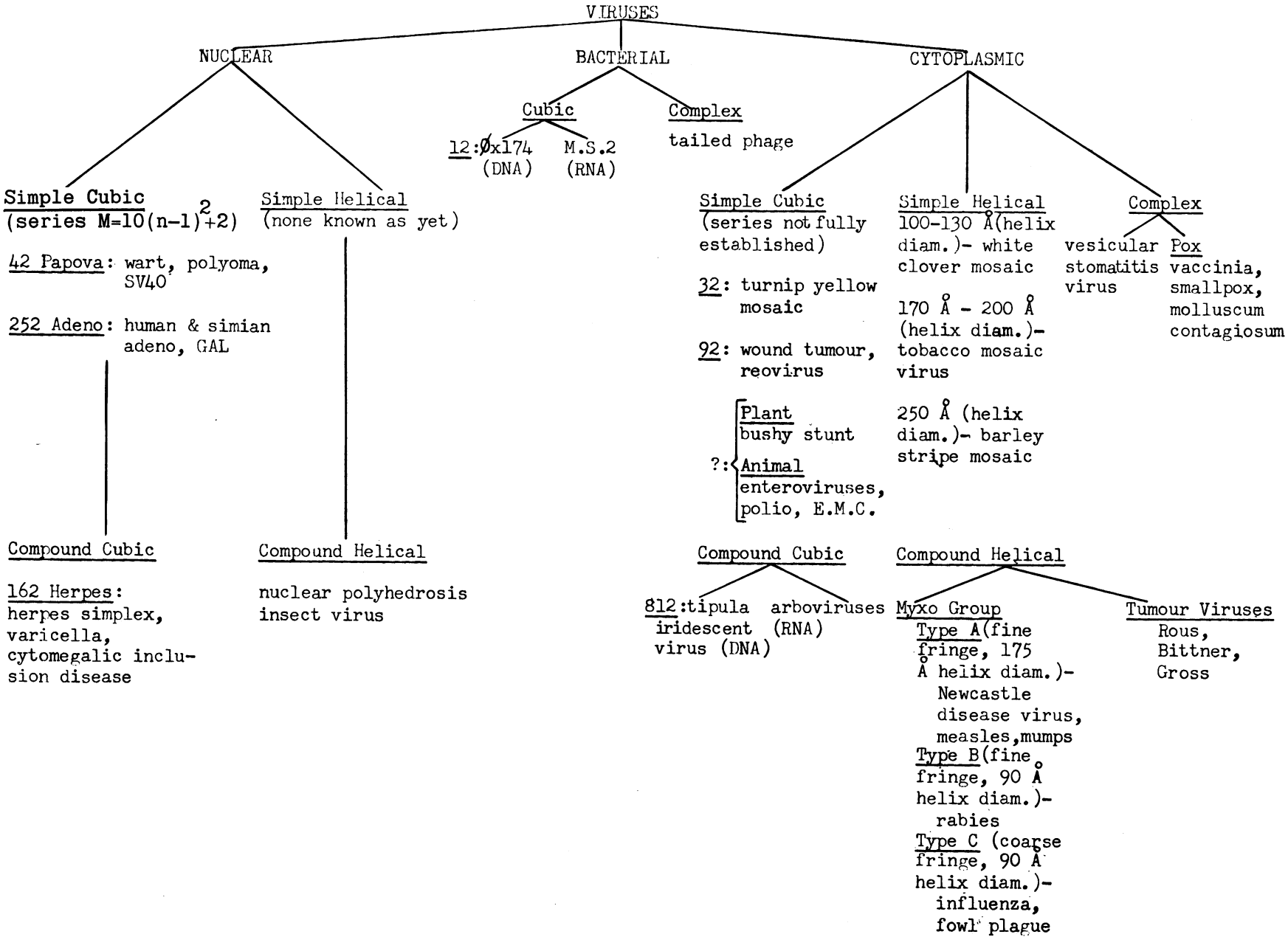


Fig. 8.—A composite arrangement of electron micrographs arranged according to the proposed classification. Since all the viruses are to scale, comparative size and shape are readily seen.

TABLE I


VIRUSES
NUCLEAR
BACTERIAL
CYTOPLASMIC
Cubic
Complex
12: Øx174 (DNA)
M.S.2 (RNA)
tailed phage
Simple Cubic
(series M=10(n-1)²+2)
42 Papova: wart, polyoma, SV40
252 Adeno: human & simian adeno, GAL
Simple Helical
(none known as yet)
Simple Cubic
(series not fully established)
32: turnip yellow mosaic
92: wound tumour, reovirus
?: Plant bushy stunt
Animal enteroviruses, polio, E.M.C.
Simple Helical
100-130 Å (helix diam.)- white clover mosaic
170 Å - 200 Å (helix diam.)- tobacco mosaic virus
250 Å (helix diam.)- barley stripe mosaic
Complex
vesicular stomatitis virus
Pox vaccinia, smallpox, molluscum contagiosum
Compound Cubic
162 Herpes: herpes simplex, varicella, cytomegalic inclusion disease
Compound Helical
nuclear polyhedrosis insect virus
Compound Cubic
812: tipula iridescent virus (DNA)
arboviruses (RNA)
Compound Helical
Myxo Group
Type A (fine fringe, 175 Å helix diam.)- Newcastle disease virus, measles, mumps
Type B (fine fringe, 90 Å helix diam.)- rabies
Type C (coarse fringe, 90 Å helix diam.)- influenza, fowl plague
Tumour Viruses
Rous, Bittner, Gross

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CLASSIFICATION

In the light of the foregoing we are now in a position to consider how information derived from a study of viruses with the electron microscope could serve to provide the basis for a useful classification of viruses. First, the study of thin sections permits viruses to be classified as to whether or not they multiply in the nucleus or the cytoplasm of the cell. Secondly, negative staining permits their fine structure to be determined and hence their allocation into categories which relate to the type of symmetry they manifest. By combining these two criteria we then can arrange the viruses into a classification as shown in Table I* and illustrated in Fig. 8.

The organization of the proposed classification shown in Table I and Fig. 8 is therefore as follows. The first distinction made is whether or not any particular virus multiplies in the nucleus or cytoplasm of the cell. This gives rise to two main divisions. However, since the bacterial viruses cannot be classified in this way they have to be set in a special category, so that there are three main categories in all.

Next, within each of these three categories the members are further classified as to whether they manifest cubic, helical, or complex symmetry. The next distinction that can be made relates to those categories where there are viruses that, although manifesting the same type of symmetry, differ because some are surrounded by an outer membrane and some are not; this gives rise to the distinction that permits them to be classified as compound or simple. Still further characterization can be provided by the number of morphological subunits present in the protein shell of those viruses having cubic symmetry. The viruses having helical symmetry can also be classified still further by the nature of the helix and, in the instance of the compound helical viruses, by the morphological features of the surrounding membrane.

It might now be asked whether groups of viruses that have morphological features in common have any other properties which they also share in common. In other words, now that so much is known about the structure of viruses, it might be asked if there is any relation between certain structural features and biochemical properties. In this connection it is of interest that a recent classification of Hamparian, Hilleman and Ketler,[21] based on biochemical and physical properties of viruses, is arranged into groupings very similar to those shown here; for example, the group described in this classification as simple cubic nuclear becomes in its entirety the acid- and ether-stable DNA group of their classification. Compound cubic nuclear of this classification matches the acid- and ether-labile DNA group. Simple cubic cytoplasmic matches their heat-labile, ether- and acid-stable RNA group. Compound helical cytoplasmic viruses in general match their acid- and ether-labile RNA group. In no case did a group placed together on morphological grounds disagree with groups placed together on other bases and physical behaviour.

It is also of interest that when grouped in the manner proposed it becomes apparent that tumour-inducing activity is associated with two main groups of viruses, the simple cubic nuclear and the compound helical cytoplasmic. This will be discussed in somewhat more detail in the accompanying article by McLeod and Ham.[22]

If it is true, then, that an arrangement based on morphology can give us information about other aspects of a virus or even cut down the range of possibilities to be tested, then a morphological basis of classification has much to offer from a purely practical point of view. Since thin sectioning is a standard technique wherever there are electron microscopes, and negative staining of the type mentioned here is a straightforward technique which makes virus purification unnecessary, the position in a morphological classification of any virus that is under study can be readily determined.

*Table I is by no means exhaustive and lists only some of the commoner viruses.

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